

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): FRENCH, Gary L., et al.

Atty. Docket: 78104.028

Title: IDENTIFICATION OF BACTERIA BY AMPLIFICATION AND PROBING

**"CLEAN" CLAIMS AS AMENDED, 37 CFR §1.121(c)(1)(i)**

39. (New) A method for identifying bacteria in a test sample, the method comprising:
- (a) amplifying a portion of 23S rDNA present in the test sample using a primer pair comprising a first primer comprising one or more oligonucleotides having a sequence as shown in SEQ. ID. NO: 1; and a second primer comprising an oligonucleotide having a sequence as shown in SEQ. ID. NO: 2, to thereby yield amplicons; and then
  - (b) probing with the amplicons of step (a) oligonucleotides designed to identify bacteria which may be present in the sample, wherein selective hybridization of the amplicons to the oligonucleotides indicates the identity of the bacteria present in the sample.
40. (New) The method according to Claim 39, wherein in step (b) oligonucleotides designed to identify at least eight bacterial species are probed.

41. (New) The method according to Claim 39, in which are probed oligonucleotides designed to identify at least one of *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus spp.*, *Klebsiella spp.*, *Enterobacter spp.*, *Proteus spp.*, *Pneumococci*, and coagulase-negative *Staphylococci*.
42. (New) The method according to Claim 39, wherein in step (b) oligonucleotides designed to identify at least ten bacterial species are probed.
43. (New) The method according to Claim 39, in which are probed oligonucleotides designed to identify at least one of *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Enterococcus faecium*, *Enterococcus faecalis*, *Staphylococcus aureus*, coagulase-negative *Staphylococcus*, *Listeria spp.*, *Stenotrophomonas maltophilia*, *Burkholderia cepacia*, and *Escherichia coli*.
44. (New) The method according to Claim 39, in which the oligonucleotides have sequences selected from the group consisting of SEQ. ID. NOS: 3-7, 9-13, 15-19, 21-28, 30-32, 39-41, 44-49, 51, and 53-58.
45. (New) The method according to Claim 39, in which the oligonucleotides have sequences selected from the group consisting of SEQ. ID. NOS: 8, 14, 20, 29, 33-38, 42, 43, 50, 52, and 59.
46. (New) The method according to Claim 39, in which the oligonucleotides have sequences selected from the group consisting of SEQ ID NOS: 3-59.

47. (New) The method according to Claim 39, in which the oligonucleotides have sequences selected from the group consisting of SEQ ID NOS: 60-63.
48. (New) The method according to Claim 39, wherein in step (a), the 23S rDNA present in the test sample is amplified by the polymerase chain reaction (PCR).
49. (New) The method according to Claim 39, wherein in step (a), the 23S rDNA present in the test sample is amplified by transcription mediated amplification.
50. (New) The method according to Claim 39, in which the oligonucleotides are attached to a support material.
51. (New) A primer pair comprising a first primer comprising one or more oligonucleotides having a sequence as shown in SEQ. ID. NO: 1; and a second primer comprising an oligonucleotide having a sequence as shown in SEQ. ID. NO: 2.
52. (New) The primer pair according to Claim 51, wherein one of the first primer or the second primer is labeled.
53. (New) The primer pair according to Claim 51, wherein one of the first or second primers is labeled with digoxigenin.

54. (New) A set of oligonucleotides for identifying specific bacteria present in a test sample, the set comprising a plurality of oligonucleotides designed to identify simultaneously different bacterial species which may be present in a test sample, the oligonucleotides being capable of specifically hybridizing to a segment of bacterial 23S ribosomal nucleic acid amplified using primers having a sequence as shown in SEQ. ID. NOS: 1 and 2.
55. (New) A set of oligonucleotides having sequences selected from the group consisting of SEQ. ID. NOS: 3-7, 9-13, 15-19, 21-28, 30-32, 39-41, 44-49, 51, and 53-58.
56. (New) A set of oligonucleotides having sequences selected from the group consisting of SEQ. ID. NOS: 8, 14, 20, 29, 33-38, 42, 43, 50, 52, and 59.
57. (New) A set of oligonucleotides having sequences selected from the group consisting of SEQ. ID. NOS: 3-59.
58. (New) A set of oligonucleotides having sequences selected from the group consisting of SEQ. ID. NOS: 60-63.
59. (New) A set of oligonucleotides having sequences selected from the group consisting of SEQ. ID. NOS: 1-63, the oligonucleotides being affixed to a support substrate.
60. (New) The set of oligonucleotides according to Claim 59, in which some or all of the oligonucleotides are affixed to the support by means of one or more chemically-modified or added nucleotide bases.

61. (New) The set of oligonucleotides according to Claim 60, wherein at least one additional thymine base is affixed to each individual oligonucleotide at its 3' end.
62. (New) A diagnostic kit for the identification of bacteria, the kit comprising an amplification primer pair having a first primer and a second primer, each first primer having a sequence selected from the group consisting of SEQ. ID. NOS: 1 and 2, and each second primer having a sequence selected from the group consisting of SEQ. ID. NOS: 3-63.
63. (New) The diagnostic kit according to Claim 62, wherein the first and second primers are affixed to a support substrate.
64. (New) The diagnostic kit according to Claim 62, wherein one of the first primer or the second primer is labeled.
65. (New) The diagnostic kit according to Claim 62, wherein one of the first or second primers is labeled with digoxigenin.
66. (New) An isolated oligonucleotide having a sequence which is any one of the sequences from SEQ. ID. NO: 3 to SEQ. ID. NO: 63, inclusive.
67. (New) An isolated oligonucleotide according to Claim 66, having a sequence as shown in SEQ ID. NOS: 3 or 4.
68. (New) An isolated oligonucleotide according to Claim 66, having a sequence as shown in SEQ. ID. NOS: 5, 8, 10, 37, or 48.

69. (New) An isolated oligonucleotide according to Claim 66, having a sequence as shown in SEQ. ID. NOS: 6 or 7.
70. (New) An isolated oligonucleotide according to Claim 66, having a sequence as shown in SEQ. ID. NOS: 9, 38, 49.
71. (New) An isolated oligonucleotide according to Claim 66, having a sequence as shown in SEQ. ID. NO: 13.
72. (New) An isolated oligonucleotide according to Claim 66, having a sequence as shown in SEQ. ID. NOS: 16 or 19.
73. (New) An isolated oligonucleotide according to Claim 66, having a sequence selected from the group consisting of SEQ. ID. NOS: 20 to 26.
74. (New) An isolated oligonucleotide according to Claim 66, having a sequence as shown in SEQ. ID. NO: 27.
75. (New) An isolated oligonucleotide according to Claim 66, having a sequence as shown in SEQ. ID. NO: 28.
76. (New) An isolated oligonucleotide according to Claim 66, having a sequence as shown in SEQ. ID. NO: 29.
77. (New) An isolated oligonucleotide according to Claim 66, having a sequence as shown in SEQ. ID. NOS: 15 or 18.